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Short Communication: Patterns of Fecal Shedding of *Klebsiella* by Dairy Cows

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ABSTRACT

Patterns and persistency of fecal shedding of *Klebsiella* spp. by healthy adult dairy cattle were explored with probabilistic, statistical, and molecular methods. Fecal shedding was monitored longitudinally in 92 animals in 1 herd for 5 mo. Shedding patterns followed a random binomial distribution, and associations with host factors were not detected. For 12 animals from 4 herds, strain-typing of multiple fecal *Klebsiella* isolates was performed by means of random-amplified polymorphic DNA typing. For 2 animals, additional typing was performed on isolates from samples collected on several consecutive days. A large variety of *Klebsiella* strains was detected within samples (on average, 3.1 strains per 4 isolates) and between samples (18 of 20 strains were detected only once in feces from cows that were sampled for 5 d consecutively). Results from each method suggest that fecal shedding of *Klebsiella* is associated with transient rather than persistent presence of the organism in the gastrointestinal tract.
Key words: *Klebsiella*, dairy cow, fecal shedding

Gram-negative bacteria are the most common etiological agents isolated from cases of clinical mastitis. The most common gram-negative bacteria causing mastitis are *Escherichia coli* and *Klebsiella* spp. (Hogan and Smith, 2003). Milk losses and mortality due to *Klebsiella* mastitis are higher compared with those resulting from *E. coli* mastitis (Gröhn et al., 2004). Vaccines and antibiotic therapy have limited effect against *Klebsiella* mastitis (Hogan and Smith, 2003; Roberson et al., 2004). Therefore, *Klebsiella* mastitis control is primarily based on prevention. Use of inorganic bedding material and good bedding management are the main preventive measures (Kristula et al., 2005). A recent study showed that fecal shedding of *Klebsiella* could occur in as many as 80% of healthy dairy cows (Munoz et al., 2006). This suggests that fecal shedding of the organism may contribute to the exposure of cows to *Klebsiella* and to occurrence of mastitis. For some pathogens, for example, *Salmonella* Dublin (Nielsen et al., 2004) and *Mycobacterium avium* spp. *paratuberculosis* (Van Schaik et al., 2005), fecal shedding plays an important role in disease dynamics. The identification and removal of carriers is used in control strategies for these pathogens. For other pathogens such as *Listeria monocytogenes* (Nightingale et al., 2004) or *Streptococcus uberis* (Zadoks et al., 2005), fecal shedding is not persistent and its role in disease transmission is not clear. The aim of this study was to explore whether fecal shedding of *Klebsiella pneumoniae* by dairy cows is intermittent, suggesting transient presence or passthrough of organisms, or persistent, which would suggest a gastrointestinal carrier state. To this end, shedding patterns were analyzed with probabilistic methods, associations between shedding status and host factors were evaluated with statistical methods, and heterogeneity of fecal *Klebsiella* isolates was determined with molecular methods.

A 5-mo longitudinal study was conducted from June through October 2005 on a commercial dairy farm in upstate New York, with approximately 1,200 Holstein milking cows housed in a freestall facility. Two cohorts of 50 cows each were selected from two 200-cow pens with sand bedding. The cows were selected based on the expectation that they would be in the same mid to late lactation group for the next 5 mo. Individual fecal samples from each cow were collected once a month directly from the rectum using individual palpation sleeves (Munoz et al., 2006). Samples were transported in cooler boxes with ice packs and arrived at the laboratory within 1 h after the 3-h collection ended. Samples were evaluated for presence of *Klebsiella* spp. within 24 h of arrival (Munoz et al., 2006). Briefly, a 1:10 dilution of fecal matter in saline was incubated for 4 h at 37°C, and streaked onto MacConkey agar containing 10 mg/L of ampicillin. Species identity of colonies with *Klebsiella* morphology was confirmed using citrate, motility, and indole testing (National Mastitis Council, 1999). A cow was considered to shed *Klebsiella* if at least 1 colony of *Klebsiella* was detected.

Shedding patterns were evaluated to determine whether shedding of *Klebsiella* could be described by a

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purely random binomial process, or whether extrabinomial variation was present. The latter would suggest that some cows were more likely to consistently shed *Klebsiella* than others, potentially due to carrier and noncarrier states. The binomial distribution was given by:

$$P(FS^{+}; n, p) = \frac{n!}{FS^{+}!(n - FS^{+})!} p^{FS^{+}}(1 - p)^{n-FS^{+}}$$

where FS^{+} is the number of fecal samples testing positive for *Klebsiella* (ranging from 0 to 5), n is the number of samples for an individual cow, p is the prevalence of shedding, and $P(FS^{+})$ is the probability of finding FS^{+} positive samples for a cow. Observed and expected values for the number of *Klebsiella*-positive samples per cow were cross tabulated and the differences between observed and expected values were evaluated using Fisher's exact test. The goals were to determine the probability of finding a specified number of positive samples and the probability of finding specific patterns of shedding. The prevalence of fecal shedding within the cohort differed numerically between samplings (Table 1). As a result, the probability of finding a specified number of positive samples differed between patterns. For example, 3 positive samples could be found in the combinations (P, P, N, P, N) and (N, N, P, P, P), where P represents a positive sample and N represents a negative sample. The expected frequency of the first pattern would be $[0.76 \times 0.77 \times (1 - 0.86) \times 0.82 \times (1 - 0.84)] = 1\%$, and the expected frequency of the second pattern would be $[(1 - 0.76) \times (1 - 0.77) \times 0.86 \times 0.82 \times 0.84) = 3\%$.

Data on DIM, milk yield, SCC, and parity were collected from herd records maintained in DairyComp 305 (Valley Agricultural Software, Tulare, CA). Data on DIM, yield, and SCC from test days were compared with fecal shedding status for the sampling that was closest in time, either before or after the test day. The distribution of continuous variables (log transformed SCC, DIM, milk yield) was explored using basic descriptive statistics to identify outliers and nonlinearity of associations. Differences in cow factors were compared between *Klebsiella*-positive and negative animals using 2-sided 2-sample t-tests. Parity was treated as a categorical variable and associations between shedding status and parity were tested using Pearson's χ^2 statistic. Statistical analyses were conducted with Splus version 6.2 (Insightful Corp., Seattle, WA). Significance level was set at $P < 0.05$.

Fecal samples were collected from the rectum of a convenience sample of 10 healthy dairy cows in 4 herds (A, B, C, and D). Samples were processed as described above. Up to 4 colonies per fecal sample were selected for confirmation as *Klebsiella*. The number of samples yielding 4 confirmed *Klebsiella* colonies for subsequent strain typing ranged from 1 to 6 per herd. In 1 herd (D), longitudinal follow up was performed for 2 cows (D1 and D2) that were *Klebsiella* positive upon initial screening. Additional samples were collected from cows D1 and D2 for 5 d consecutively, 3 wk after the initial sampling, and processed in the same manner as the fecal samples from herds A through D. *Klebsiella* isolates were further characterized by random amplified polymorphic DNA (**RAPD**) PCR using primer set AP4/ERIC-1R: 5′-TCACGATCGA-3′/5′-ATGTAAGCTCCTGGGGATTCGC-3′ (Barbier et al., 1996), or ERIC-2/ERIC-1026: 5′-AAGTAAGTGACTGGGGTGAGCG-3′/5′- TACATTCGAGGACCCCTAAGTG-3′ (Vogel et al., 1999). Primers were obtained from Integrated DNA Technologies (Coralville, IA). Lysates for PCR were prepared using 10-min boil preparation of cell pellets suspended in 1× Tris-EDTA buffer (Promega, Madison, WI). Cycling conditions for primer sets AP4/ERIC-1R were initial denaturation for 4 min at 94°C; 44 cycles of 30 s at 94°C, 1 min at 35°C, and 2 min at 72°C; and final extension for 7 min at 72°C (Barbier et al., 1996).

Table 1. Observed and expected frequency of *Klebsiella* shedding patterns in monthly fecal samples from 92 dairy cows[1]

Sampling					Pattern frequency[2]	
1	2	3	4	5	Observed	Expected[3]
+	−	−	−	−	1	0
−	+	+	−	−	1	0
+	+	+	−	−	1	1
−	+	−	+	−	1	0
+	+	−	+	−	0	1
−	−	+	+	−	1	1
+	−	+	+	−	2	2
−	+	+	+	−	2	2
+	+	+	+	−	5	6
+	+	−	−	+	2	1
−	−	+	−	+	2	1
+	−	+	−	+	1	2
−	+	+	−	+	2	2
+	+	+	−	+	7	7
−	−	−	+	+	2	0
+	−	−	+	+	2	2
−	+	−	+	+	1	2
+	+	−	+	+	4	5
−	−	+	+	+	0	3
+	−	+	+	+	10	10
−	+	+	+	+	10	10
+	+	+	+	+	35	32

[1]Patterns that were not observed and for which <1 cow was expected are not shown; plus and minus signs indicate positive and negative test results, respectively.

[2]Observed and expected values add to 92 and 90, respectively. Differences in total numbers are due to rounding off of expected values to whole numbers and omission of patterns with <1 expected animals and zero observed animals.

[3]Expectation based on assumption of independence of observations and shedding prevalence of 76, 77, 86, 82, and 85%, respectively, at samplings 1 through 5.